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Maternal and paternal transmission to offspring of B chromosomes of *Zea mays* L. in the alien genetic background of *Avena sativa* L.

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B chromosomes (Bs) are supernumerary dispensable chromosomes with highly host-specific organization, behavior and mode of inheritance described in hundreds of animal, fungal and plant species. We transferred native Bs of maize (*Zea mays* L. ssp. *mays* cv. Black Mexican Sweet) to oats (*Avena sativa* L. ssp. *sativa* cv. Starter) (Kynast et al., MNL 81:16, 2007) since native Bs of oats have not been reported to exist in wild and cultivated populations of hexaploid oat species. However, native Bs of maize belong to the first-discovered (Kuwada, Bot. Mag. Tokyo 39:227-234, 1925), and presumably molecularly and cytogenetically best-described (Jones and Diez, The B chromosome database, http://www.bchromosomes.org/bdb/, 2004), Bs in the plant realm. Among their extraordinary features of structure and function, native Bs of maize are capable of prevailing in populations by balancing selfish drive and counteracting factors which are genetically controlled by different genes/factors that have been assigned to the Bs themselves, as well as to the host genome. We address the question in our research objectives: How will a native B of maize behave after being converted into an alien B by transferring it into hexaploid oats – a very remotely related species that has not been exposed to a native B during its entire evolution?

Hybridization experiments of the three common oat cultivars Starter, Sun II and Paul (2n = 6x = 42, *Avena sativa* L. ssp. *sativa*) by the maize line B73^{B} – a dent corn inbred B73 derivative that carries six Bs of the sweet corn cultivar Black Mexican Sweet (2n = 2x + 6$_B$ = 26) generously provided by J. A. Birchler, University of Missouri-Columbia – generated 14 F1-plants with complete sets of 21 oat chromosomes and different numbers of individual maize chromosomes, resulting from incomplete uniparental genome loss (UGL) during early stages of the F1-plants' embryogeneses. The retained maize chromosomes were found in shoot tissues based on PCR results for *Grande-1*, a dispersed LTR-type retrotransposon, which is abundant on all A-chromosomes (As) and Bs of maize but absent from all chromosomes of the three oat genotypes used in our crossing program. Two of these 14 F1-plants (5811_1 and 5845_1) proved to carry maize Bs in shoot and root tissues. PCR assays involving two B-specific markers (primer pair p-2ndb1 + p-2ndb4 and primer pair p-brpt2 + p-taralb1, generously provided by J. A. Birchler) and a selected set of A-specific markers for maize (chromosome arm-specific SSR markers selected from the 'Maize Genetics and Genomics Database', http://www.maizegdb.org/) showed that in both plants the *Grande-1*-positive PCR products resulted from the presence of maize Bs and the absence of maize As (Figure 1). Cytological analyses by the use of fluorophore-labeled genomic DNA of maize in GISH assays on primary root meristems of very young, juvenile plantlets revealed that in the F1-plant 5811_1 all ten maize As were eliminated, and three maize Bs were retained along with the complete set of 21 oat chromosomes (2n = 3x+3$_B$ = 24). In the primary root meristem of the F1-plant 5845_1 all ten maize As were eliminated, and a single maize B was retained along with the complete set of 21 oat chromosomes (2n = 3x+1$_B$ = 22).

Self-pollination of the F1-plants 5811_1 and 5845_1 has produced up to this point, a total of 132 F2-seeds in both genotypes due to frequent formation of unreduced female and male gametes (Table 1). Partial fertility had already been observed in haploids of Starter, Sun II and Paul oats without (Rines et al., In: Jain, Sopory, Veilleux (eds) Kluwer Acad Publishers, Dordrecht, The Netherlands, In vitro haploid production in higher plants 4, pp. 205-221, 1997) and with (Kynast et al., PNAS 101:9921-9926, 2004) the addition of individual As of B73 maize. Cytological and molecular analyses of 30 F2-offspring plants showed that the F1-plant 5811_1 – carrying three Bs – produced six F2-plants each without Bs (2n = 6x = 42), three F2-plants each with one B (2n = 6x + 1$_B$ = 43), nine F2-plants each with two Bs (2n = 6x + 2$_B$ = 44), one F2-

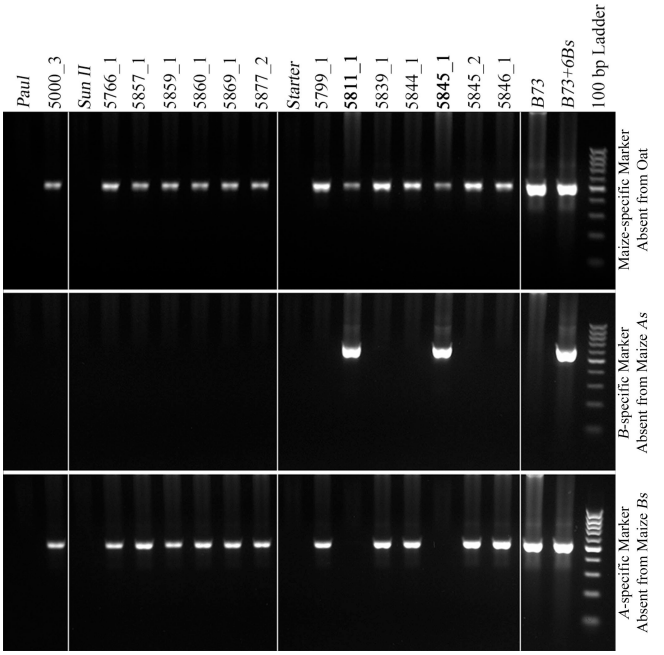

Figure 1. PCR products from genomic DNA of three oat plants, 14 F1 (oat × maize) plants, one maize plant without B, and one maize plant with six Bs by using a maize-specific, a B-specific and an *A*-specific marker; electrophoresis in 1.5% agarose

Table 1. Plant material for crossing three different oat cultivars (2n = 6x = 42) by the maize B73^{B} (2n = 2x+6$_B$ = 26) and results of maize *B*-positive offspring production.

Oat cultivars	Starter	Sun II	Paul
Oat panicles	40	53	3
Oat florets, emasculated and hand-pollinated	1177	1094	70
F1-proembryos, *in vitro* rescued 14-15 days after pollination	62	52	1
F$_1$-embryos, geminated*	14	16	1

Maize (A and/or B)-positive juvenile F1-plantlets (shoot- and root-tested)	7	6	1
Maize B-positive adult F1-plants (shoot- and root-tested)	2	0	0
F1 5811_1** (tiller-tested)	1	n/a	n/a
F1 5845_1** (tiller-tested)	1	n/a	n/a
Total F2-offspring of F1 5811_1, harvested to date	59	n/a	n/a
Total F2-offspring of F1 5845_1, harvested to date	73	n/a	n/a
Maize B-positive / Tested F2-offspring of F1 5811_1 (shoot- and root-tested)	24 / 30	n/a	n/a
Maize B-positive / Tested F2-offspring of F1 5845_1 (shoot- and root-tested)	0 / 30	n/a	n/a

*Embryos that formed shoot and root with enough tissue for molecular and cytogenetic analyses; **Plants represent clonal tillers from two clones after extensive tiller cloning allowing for more F2-seed production

plant with three Bs (2n = 6x + 3_B = 45), two F2-plants each with four Bs (2n = 6x + 4_B = 46), and nine F2-plants with highly chimeric root meristems showing cells with one to five Bs (2n = 6x + 1_B … 5_B = 43 … 47) in different frequencies (Figure 2). In contrast, none of the 30 F2-offspring of the F1-plant 5845_1 – carrying one B – had Bs based on the results of cytological and molecular tests (Table 1). Taking all data of the F1 and F2 analyses together, our results show that (1) maize Bs can be added to the complete haploid genome of oats via inter-species (oat × maize) hybridization and successive incomplete UGL, (2) haploid oat plants hosting one or three maize Bs are partially fertile, mainly because of frequent formation of unreduced gametes of both sexes, and (3) maize Bs can be transmitted to F2-offspring, which has been observed as being doubled haploid (=hexaploid) oat plants without and with the addition of one to four Bs, and occasionally up to five Bs in chimeric root meristems.

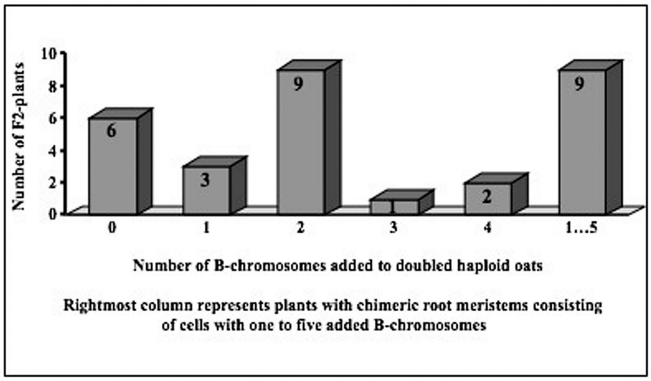

Figure 2. Numbers of F2-plants without and with an added maize B chromosome.

However, the transmission of added maize Bs from haploid F1-oats to doubled haploid (=hexaploid) F2-oats is very special due to two particularities: Firstly, the frequency of transmission from F1 to F2 does not correlate with the frequency of retention during the incomplete UGL process in the primary inter-species hybrid. Secondly, the transmission from F1 to F2 does not necessarily reflect alien B transmission in general due to the meiotic restitution process leading to unreduced gametes and doubled haploid F2-plants. Therefore, transmission rates from F1 to F2 apparently do not allow transmission rates being predicted for successive offspring generations. Thus, transmission of maize Bs added to oats was analyzed also in F3- and BC1-offspring. Investigations of F3-offspring of three selected F2-plants with one, two, and three added maize Bs to their oat genomes showed that the alien Bs neither became immediately eliminated from the oat genome nor became excessively accumulated in the oat genome (Table 2). The frequencies of B transmission from F2 to F3 did not correspond with Mendelian expectation values for chromosome segregation common for As in monosomic, disomic, and trisomic condition with regular meiotic behavior. For instance, the offspring of the trisomic B addition F2 1188_20 generated only monosomic and disomic B additions indicating a tendency of B loss. However, the F3-offspring 1390_2 of the disomic B addition F2 1188_19 "gained" one B by becoming a trisomic B addition. This accumulation certainly indicates irregular transmission conditions. In order to characterize B transmission in more detail, we backcrossed F3-plants with monosomic and disomic B addition (male parent) to Starter oat (female parent). Both offspring populations showed successful B transmission. Eight tested BC1-offspring descending from the monosomic B addition accounted for two euploid oat

Table 2. Chromosome numbers of F3-offspring plants descended from three F2-plants with one, two, and three added B chromosomes.

F2-Genotype	2n =	F3-Offspring	2n =
1188_21	6x + 1_B = 43	1392_1	6x + 0_B = 42
		1392_2	6x + 0_B = 42
		1392_3	6x + 0_B = 42
		1392_4	6x + 1_B = 43
1188_19	6x + 2_B = 44	1390_1	6x + 2_B = 44
		1390_2	6x + 3_B = 45
		1390_3	6x + 1_B = 43
		1390_4	6x + 1_B = 43
1188_20	6x + 3_B = 45	1391_1	6x + 1_B = 43
		1391_2	6x + 0_B = 42
		1391_3	6x + 2_B = 44
		1391_4	6x + 2_B = 44

plants, three monosomic and three disomic B addition plants. Besides the herewith proven paternal transmission of maize Bs in an oat background, the 3/8 frequency of BC1-genotypes with an increased number of Bs demonstrates the competitive strength of male oat gametes hosting maize Bs and a tendency to prevail similar to the situation in maize – the native host species. Among eight tested BC1-offspring descending from the disomic B addition, three plants were euploids, four plants were monosomic B additions, and one plant was a tetrasomic B addition. B accumulation took place, although at a low 1/8 frequency. In order to test for maternal maize B transmission in the oat background, we backcrossed F2-plants with a disomic B addition (female parent) by Starter oat (male parent). Among six tested BC1-offspring, three plants were euploid oats and three plants had monosomic B additions. These data prove that maize Bs can be maternally transmitted in oats. However, the limited number of offspring tested to date does not show whether female sporogenesis and/or megagametogenesis may also accumulate maize Bs in an oat background. Thus, further offspring genotypes are being characterized. Analyses of meiosis and gametogenesis are in progress.